

Gas exchange and photosynthetic acclimation over subambient to elevated CO₂ in a C₃–C₄ grassland

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Abstract

Atmospheric CO₂ (C_a) has risen dramatically since preglacial times and is projected to double in the next century. As part of a 4-year study, we examined leaf gas exchange and photosynthetic acclimation in C₃ and C₄ plants using unique chambers that maintained a continuous C_a gradient from 200 to 550 µmol mol^{–1} in a natural grassland. Our goals were to characterize linear, nonlinear and threshold responses to increasing C_a from past to future C_a levels. Photosynthesis (A), stomatal conductance (g_s), leaf water-use efficiency (A/g_s) and leaf N content were measured in three common species: *Bothriochloa ischaemum*, a C₄ perennial grass, *Bromus japonicus*, a C₃ annual grass, and *Solanum dimidiatum*, a C₃ perennial forb. Assimilation responses to internal CO₂ concentrations (A/C_i curves) and photosynthetically active radiation (A/PAR curves) were also assessed, and acclimation parameters estimated from these data. Photosynthesis increased linearly with C_a in all species ($P < 0.05$). *S. dimidiatum* and *B. ischaemum* had greater carboxylation rates for Rubisco and PEP carboxylase, respectively, at subambient than superambient C_a ($P < 0.05$). To our knowledge, this is the first published evidence of A up-regulation at subambient C_a in the field. No species showed down-regulation at superambient C_a. Stomatal conductance generally showed curvilinear decreases with C_a in the perennial species ($P < 0.05$), with steeper declines over subambient C_a than superambient, suggesting that plant water relations have already changed significantly with past C_a increases. Resource-use efficiency (A/g_s and A/leaf N) in all species increased linearly with C_a. As both C₃ and C₄ plants had significant responses in A, g_s, A/g_s and A/leaf N to C_a enrichment, future C_a increases in this grassland may not favour C₃ species as much as originally thought. Non-linear responses and acclimation to low C_a should be incorporated into mechanistic models to better predict the effects of past and present rising C_a on grassland ecosystems.

Keywords: subambient CO₂, elevated CO₂, photosynthetic acclimation, up-regulation, photosynthesis, stomatal conductance, resource-use efficiency, grassland

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Introduction

Considerable research has been devoted to understanding physiological responses of plants to future atmos-

pheric CO₂ (C_a) increases and the resulting consequences for natural and agricultural ecosystems (reviewed in Drake *et al.* 1997; Curtis & Wang 1998; Saxe *et al.* 1998; Hsiao & Jackson 1999; Wand *et al.* 1999; Ward & Strain 1999). Much of this work has compared plants grown at ambient C_a (360 µmol mol^{–1}) with those grown at twice ambient concentrations, and has shown that increased C_a enhances photosynthesis (A) and growth, decreases

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stomatal conductance (g_s), and increases leaf water-use efficiency (A/g_s) for a variety of species and ecosystems (e.g. Owensby *et al.* 1993; Knapp *et al.* 1993; Jackson *et al.* 1994, 1995). However, nonlinear or threshold responses are difficult or impossible to assess in these experiments because of the limited number of C_a concentrations examined. Recent modelling work (Ackerly & Bazzaz 1995; Luo & Reynolds 1999) and a few empirical studies at three or four C_a concentrations (e.g. Hunt *et al.* 1991, 1993) suggest that single species, community and ecosystem responses to C_a are frequently nonlinear. C_a is increasing gradually, and we cannot necessarily interpolate responses to intermediate C_a concentrations based on data at twice ambient levels. Additional experiments on plant responses over the entire C_a gradient are needed to refine our predictions of ecosystem responses to future C_a increases (Ackerly & Bazzaz 1995; Luo & Reynolds 1999).

Ambient-superambient comparisons also do not take into account past increases in C_a that have already shaped vegetation. Ice core data show that C_a fluctuated from 180 to 300 $\mu\text{mol mol}^{-1}$ during the last 250 000 years (reviewed in Sage & Cowling 1999), and was sometimes below 200 $\mu\text{mol mol}^{-1}$ for 10 000-year periods (Barnola *et al.* 1987; Jouzel *et al.* 1993). Past C_a increases have been associated with changes in the global distributions of C_3 and C_4 plants (e.g. Johnson *et al.* 1993; Ehleringer *et al.* 1997), increases in ecosystem productivity (e.g. Phillips & Gentry 1994), and the advent of agriculture (Sage 1995), but we know relatively little about the physiological responses of plants to subambient C_a , and how they compare with responses to superambient C_a . Examining plant sensitivity to a range of C_a concentrations may give insight into the physiological capacity of plants to respond to C_a increases, both past and future (Sage & Cowling 1999; Ward *et al.* 2000).

Our study examined leaf gas exchange in field-grown C_3 and C_4 plants using unique experimental chambers that maintained a continuous gradient of C_a from 200 to 550 $\mu\text{mol mol}^{-1}$ in a natural grassland community. While there has been some work with subambient C_a in crops (e.g. Baker *et al.* 1990a,b; Campbell *et al.* 1990; Rowland-Bamford *et al.* 1990; Allen *et al.* 1991; Mayeux *et al.* 1997) and wild C_3 plants in growth chambers (e.g. Overdieck 1989; Polley *et al.* 1992a,b; 1995), no studies at subambient C_a have examined intact plant communities. Furthermore, few have compared physiological responses in C_3 and C_4 species. C_4 plants are predicted to have a competitive advantage over C_3 plants at past low C_a levels, while C_a mediated increases in C_3 quantum yield are expected to favour C_3 over C_4 plants in the future (Ehleringer *et al.* 1997). The few studies at subambient C_a using both C_3 and C_4 plants found reduced growth, A and A/g_s , and increased g_s for C_3

species (Polley *et al.* 1992b, 1993; Dipperry *et al.* 1995; Tissue *et al.* 1995). In contrast, the C_4 plants had minimal growth responses to low C_a , but some showed increased g_s and reduced A/g_s . The growth results are consistent with the expectation that A is less sensitive to C_a in C_4 than C_3 plants (but see Wand *et al.* 1999 and Ghannoum *et al.* 2000).

Although A is usually stimulated by rising C_a , there is wide variation in species' responses. One source of this variation is acclimation of A to C_a . We define acclimation as a physiological adjustment to a given C_a , particularly up- or down-regulation of A through adjustments in photosynthetic biochemistry (see Sage 1994). Assuming the balance between carbohydrate sources and sinks regulates acclimation, we might expect superambient C_a to cause down-regulation of A by increasing the source/sink ratio, and subambient C_a to cause up-regulation by decreasing the source/sink ratio (Sage & Cowling 1999). Down-regulation of A at superambient C_a is not common in the field, but is often seen in greenhouse studies (Sage 1994), possibly because sink demands are reduced by root restriction in pots (Thomas & Strain 1991). Up-regulation at subambient C_a has not been observed (Thomas & Strain 1991; Sage & Reid 1992; Tissue *et al.* 1995; Cowling & Sage 1998; but see Gesch *et al.* 2000), but has also not been studied in the field where sink demands may be greater.

In this study, we report physiological responses of grassland species to a continuous gradient of past and future C_a concentrations. We were interested in photosynthetic acclimation and comparing gas exchange responses over sub- and superambient C_a . We focused on three abundant species with different growth forms and physiological pathways: *Bothriochloa ischaemum* (L.) Keng, a C_4 perennial grass, *Bromus japonicus* L., a C_3 annual grass, and *Solanum dimidiatum* Raf., a C_3 perennial forb. We measured gas exchange parameters (A , g_s and leaf-level A/g_s) in plants along the C_a gradient over two growing seasons. We also measured assimilation responses to internal CO_2 concentrations (A/C_i curves) and photosynthetically active radiation (A/PAR curves) for each species. Acclimation and quantum yield parameters were estimated from these data to explore physiological mechanisms underlying gas exchange patterns.

Materials and methods

Study site

The experiment took place in a grassland at the USDA/ARS Grassland, Soil and Water Research Laboratory in Temple, Texas, USA (31°05' N, 97°20' W). The site has been managed as grassland for at least 50 years, and was last grazed by cattle in 1992. The vegetation was a diverse

Table 1 Measurement dates and LI-6400 leaf chamber conditions for three species along the C_a gradient. It was not logistically possible to measure plants in all sections of the experimental chambers on one day. Therefore, on each day plants at different points along the C_a gradient were randomly selected for gas exchange measurements, assuring that a wide range of the gradient was represented. Data from different days of a given measurement session (e.g. 8–13 April 1999) were pooled to characterize the gas exchange responses of a species at that point in the season. 'AC' and 'AP' indicate dates when A/C_i and A/PAR curves, respectively, were measured. LAVPD = leaf-to-air vapour pressure deficit. The leaf temperatures and LAVPDs maintained in the LI-6400 chamber reflect the different times of year and environmental conditions during which species were active.

Species and LI-6400 chamber conditions	Spring measurement dates	Summer measurement dates
<i>Bromus japonicus</i> (C ₃ annual grass) Saturating irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$): 1200 Leaf temperature (°C): 20–23 LAVPD (kPa): 1.1–1.5 in 1999 0.9–1.1 in 2000	8–13 April 1999, AC 20–25 April 2000, AP	No plants present
<i>Solanum dimidiatum</i> (C ₃ perennial forb) Saturating irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$): 1600 Leaf temperature (°C): 27–30 LAVPD (kPa): 0.9–1.1		24–27 June 1999, AP 8–14 July 1999, AC 11–12 June 2000
<i>Bothriochloa ischaemum</i> (C ₄ perennial grass) Saturating irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$): 2000 Leaf temperature (°C): 29–32 LAVPD (kPa): 1.4–1.6		22–27 August 1999, AP, AC 21–30 August 2000

mixture of native and introduced grasses and forbs. Dominant plants included *B. ischaemum*, *S. dimidiatum*, and the C₃ perennial forb *Ratibida columnaris* (Sims) D. Don. Mean annual precipitation at the site is 877 mm (1913–99), and the mean maximum and minimum annual temperatures are 25.9 °C and 13.2 °C, respectively (1914–95, USDA/ARS Grassland, Soil and Water Research Laboratory weather station records). The soil is a mollisol in the Austin series (classified as a fine-silty, carbonatic, thermic, Udorthentic Haplustoll) with 35–55% clay in the top 40 cm. Inclusions of the Houston series (a vertisol defined as a fine, smectitic, thermic, Udic Haplustert) are common.

Experimental field chambers

The experimental chambers are described in detail in Johnson *et al.* (2000) and provide a continuous gradient of C_a from 200 to 360 $\mu\text{mol mol}^{-1}$ in one chamber (subambient chamber) and from 360 to 550 $\mu\text{mol mol}^{-1}$ in the other (superambient chamber). The chambers were built over parallel, adjacent plots of grassland each 60-m long, 1-m wide, and 1.5-m apart. Each chamber was divided into 10 continuous 5-m sections with chiller and condenser units connecting consecutive sections. Chambers were 1-m tall and constructed of polyethylene film, which transmitted 85–95% of incident photosynthetic photon flux density (PPFD). A large fan at the end of each chamber blew in ambient air. In the super-

ambient chamber, incoming air was enriched with CO₂ to give a concentration of 550 $\mu\text{mol mol}^{-1}$. As the air moved down each chamber, plant A reduced C_a to 360 $\mu\text{mol mol}^{-1}$ at the end of the superambient chamber and to 200 $\mu\text{mol mol}^{-1}$ at the end of the subambient chamber. As A varied with light, water and plant biomass, air flow rates in the chambers were automatically adjusted by increasing or decreasing fan speeds to accommodate A changes. At night, C_a gradients were maintained at 150 $\mu\text{mol mol}^{-1}$ above daytime levels by reversing air flow and using respiratory CO₂ releases to create the gradient.

A rubber-coated fabric barrier extended 0.9-m deep into the soil along the chamber sides. Irrigation was applied equally to each 5-m section to match rainfall outside through July 1999. After this, irrigation was applied such that soil water content in sections at ambient C_a matched that of adjacent grassland outside as measured by neutron attenuation. The total water applied to the chambers was 349 and 381 mm in the very dry years of 1999 and 2000 (up until 13 September), respectively. Atmospheric humidity (RH) and temperature (T) along the C_a gradient were re-set with chilled-water condensers placed in the 1-m area between 5-m chamber sections. Direct, instantaneous measurements of T and RH during leaf gas exchange measurements in 1998 did not show any consistent differences in these variables along the gradient (L. J. Anderson, unpublished data). Averaged across sections, daytime

air temperatures were generally 2–4 °C below ambient and were similar in the two chambers, while mean monthly vapour pressure deficits were slightly higher in the subambient chamber than in the superambient during summer (Johnson *et al.* 2000).

The chambers were activated in May (1997); and have been operating each growing season (mid-February to mid-November) since then. C_a gradients have been maintained for > 90% of growing season days, even during severe droughts (Johnson *et al.* 2000). The plastic cover was removed from the chambers for a few months each winter, when vegetation was dormant.

Steady state leaf gas exchange

Leaf gas-exchange was measured for *B. japonicus*, *S. dimidiatum* and *B. ischaemum* along the C_a gradient during the 1999 and 2000 growing seasons (Table 1). For each species, measurements were done at a minimum of six treatment C_a concentrations (six chamber sections) on three to four plants per section. Measurements were made on the youngest, fully expanded leaves with an open gas-exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) between 0900 and 1500 h central standard time. Incident irradiance during measurements was maintained at saturating levels by red-blue light-emitting diodes, and a Peltier cooling module controlled leaf temperatures (Table 1). To calculate g_s and intercellular CO_2 concentration (C_i) for the grasses, we used a boundary layer conductance of $2.84 \text{ mol m}^{-2} \text{ s}^{-1}$ for amphistomatous leaves. For *S. dimidiatum* we used a value of $1.42 \text{ mol m}^{-2} \text{ s}^{-1}$ for hypostomatous leaves. Leaf areas were measured with the LI-3000A portable leaf area meter (Li-Cor, Inc.) or from leaf dimensions, depending on sample morphology.

Apparent quantum yield and photosynthetic acclimation

Photosynthetic responses to incident irradiance (A/PAR curves) for *S. dimidiatum*, *B. ischaemum* and *B. japonicus* were measured in June 1999, August 1999 and April 2000, respectively, using the same leaves as in the survey measurements above (Table 1). To examine the effect of C_a on apparent quantum yield, we made multiple measurements at irradiances below $120 \mu\text{mol m}^{-2} \text{ s}^{-1}$, where the slope of the curve is approximately linear. The apparent quantum yield was calculated as the slope of the A/PAR curve between 10 and $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$. All A/PAR curves were measured at the treatment C_a concentration. During measurements, leaf temperatures and LAVPD were maintained as described in Table 1.

To examine photosynthetic acclimation to the C_a treatments, we measured the response of net A to calculated C_i . A/C_i curves were measured at saturating irradiances on *B. japonicus* in April, *S. dimidiatum* in July, and *B. ischaemum* in August 1999 using the same leaves as in the survey measurements (Table 1, C_a values for A/C_i curves ranged from 215 to $546 \mu\text{mol mol}^{-1}$). For the C_3 species, A/C_i curves were analysed with a mechanistic two-factor model derived by Farquhar *et al.* (1980) and modified by Harley *et al.* (1992). The light saturated rate of carboxylation (V_{cmax}) by ribulose-1,5-bisphosphate (RuBP) carboxylase (Rubisco) and maximum rate of RuBP regeneration as a function of electron transport capacity (J_{max}) were calculated using the kinetic assumptions of Harley *et al.* (1992) with the aid of software developed by S. P. Long (University of Illinois, unpublished manuscript). For the C_4 species *B. ischaemum*, the efficiency of the PEP carboxylase CO_2 pump was determined from the slope of the linear portion of the A/C_i curve. Maximum CO_2 -saturated A rates and the C_i at which A reached saturation were determined for *B. ischaemum* as in Tissue *et al.* (1995).

Leaf nitrogen and photosynthetic nitrogen-use efficiency

Leaves used for gas exchange in 1999 and leaves collected along the gradient in 1998 were dried at 65 °C to constant weight, and ground to a powder using a Crescent Wig-L-Bug (Crescent Dental, Lyons, Illinois). Powder samples were assessed for percentage C and N content using a CE Instruments NC 2100 elemental analyser (ThermoQuest Italia, Milan, Italy). Photosynthetic nitrogen-use efficiency (PNUE) was calculated for the 1999 samples as A ($\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$)/leaf N content (g m^{-2}).

Data analysis

The relationship between gas exchange variables and C_a concentration was assessed using the Regression : Curve fitting procedure in SPSS 8.0 for Windows (SPSS Inc., Chicago, IL) and the Regression Wizard in Sigma Plot 5.0 for Windows (SPSS Inc., Chicago, IL). Because ours was the first study examining physiological responses to a C_a gradient in an intact plant community, we took an exploratory approach to our data analysis and tried a diversity of models. Several different, biologically reasonable responses to the C_a gradient were observed, and these responses varied over time. Simple linear, logarithmic, power and hyperbolic functions were fitted to the data and the adjusted R^2 values compared to find the model with the best fit. When models had similar explanatory power (R^2_s within 0.05), the linear model

Table 2 Regression analysis results for relationships between C_a concentration and gas exchange parameters for three species in 1999 and 2000. NS = no significant relationship with C_a, linear = linear model ($y = ax + b$), power = power model ($y = ax^b$), log = logarithmic model ($y = \ln x + b$), hyper. 1 = hyperbolic model 1 ($y = ax/(b + x)$), hyper. 2 = hyperbolic model 2 ($y = a + (b/x^2)$), Intercept = intercept values for regression equations.

Variable, species and measurement date	Model type	Increase or decrease with C _a	Parameter value (a)	Intercept (b)	r ²	P-value
Photosynthesis (A)						
<i>B. japonicus</i> , Apr 1999	Linear	Increase	0.0322	5.22	0.60	< 0.0001
<i>B. japonicus</i> , Apr 2000	Linear	Increase	0.0294	6.16	0.69	< 0.0001
<i>S. dimidiatum</i> , Jun 1999	Linear	Increase	0.0261	9.97	0.69	< 0.0001
<i>S. dimidiatum</i> , Jun 2000	Linear	Increase	0.0293	9.71	0.61	< 0.0001
<i>B. ischaemum</i> , Aug 1999	Linear	Increase	0.0286	12.07	0.56	0.0003
<i>B. ischaemum</i> , Aug 2000	Linear	Increase	0.0302	4.02	0.68	< 0.0001
Stomatal conductance (g_s)						
<i>B. japonicus</i> , Apr 1999	Linear	Decrease	-0.8772	699.30	0.36	0.0018
<i>B. japonicus</i> , Apr 2000	Linear	Decrease	-1.0112	899.96	0.73	< 0.0001
<i>S. dimidiatum</i> , Jun 1999	Hyper. 1	Decrease	414.42	-168.4	0.68	< 0.0001
<i>S. dimidiatum</i> , Jun 2000	Hyper. 2	Decrease	208.92	9.1 × 10 ⁷	0.89	< 0.0001
<i>B. ischaemum</i> , Aug 1999	Power	Decrease	8302.9	-0.6383	0.76	< 0.0001
<i>B. ischaemum</i> , Aug 2000	Linear	Decrease	-0.1632	188.88	0.66	< 0.0001
Water-use efficiency (A/g_s)						
<i>B. japonicus</i> , Apr 1999	Linear	Increase	0.2061	-22.32	0.70	< 0.0001
<i>B. japonicus</i> , Apr 2000	Linear	Increase	0.1348	-13.75	0.87	< 0.0001
<i>S. dimidiatum</i> , Jun 1999	Linear	Increase	0.1049	-12.23	0.78	< 0.0001
<i>S. dimidiatum</i> , Jun 2000	Linear	Increase	0.1489	-27.06	0.73	< 0.0001
<i>B. ischaemum</i> , Aug 1999	Linear	Increase	0.3707	-15.53	0.82	< 0.0001
<i>B. ischaemum</i> , Aug 2000	Linear	Increase	0.4071	-26.26	0.92	< 0.0001
C_i/C_a Ratio (Mean ± SE, Range)						
<i>B. japonicus</i> , Apr 1999 (0.74 ± 0.02, 0.61–0.85)	Linear	Decrease	-0.0004	0.89	0.45	0.0010
<i>B. japonicus</i> , Apr 2000 (0.81 ± 0.01, 0.73–0.86)	Linear	Decrease	-0.0002	0.88	0.48	0.0015
<i>S. dimidiatum</i> , Jun 1999 (0.81 ± 0.01, 0.76–0.86)	Linear	Decrease	-0.0001	0.85	0.23	0.0436
<i>S. dimidiatum</i> , Jun 2000 (0.82 ± 0.01, 0.72–0.89)	Linear	Decrease	-0.0002	0.90	0.36	0.0038
<i>B. ischaemum</i> , Aug 1999 (0.35 ± 0.02, 0.25–0.48)	Linear	Decrease	-0.0003	0.47	0.31	0.0198
<i>B. ischaemum</i> , Aug 2000 (0.42 ± 0.01, 0.32–0.55)	Linear	NS	-0.0002	0.50	0.12	0.0685

was selected. Plots of residuals were examined for normality and homoscedasticity for all linear fits, and no violations of assumptions were found. As several measurements were taken within a given section of the C_a gradient, we generally had more than one y -value for a given x (where $x = C_a$ concentration). Therefore we tested the hypothesis that the data were linear using the Lack-of-Fit option in the General Linear Model procedure in SPSS 8.0 and following the guidelines in Zar (1996) for regression with replication. For clarity, y -values in figures are presented as means with standard errors, but all analyses were done on individual variates. In cases where there was no significant relationship with C_a, data are plotted with a linear model. Additional lines were

hand-fit to the quantum yield data to highlight patterns not well described by the overall models for each data set. Data for each species at each time point were analysed separately (Tables 2–4), although 1999 and 2000 data described by similar models are plotted with a single line in the figures, to emphasize the consistency of the relationship with C_a.

Results

Patterns of gas exchange along the CO₂ gradient

Photosynthetic rates increased significantly with increasing C_a for all three species in 1999 and 2000, more than 2 years after C_a treatments were initiated. All responses

were linear across sub- and superambient concentrations ($P < 0.05$, Fig. 1, Table 2). The relationship between A and C_a was similar for 1999 and 2000 in *B. japonicus* and *S. dimidiatum* (Fig. 1A, B). For *B. ischaemum*, the slope of the relationship was similar for the 2 years, although the absolute magnitude of A was 35% lower on average in 2000 than in 1999, likely due to the severe drought in the summer of 2000 (Fig. 1C).

Stomatal conductance decreased significantly with increasing C_a for all species in both years (Fig. 1, Table 2). *Solanum dimidiatum* showed strong curvilinear responses on both measurement dates, with larger declines in g_s across subambient than superambient C_a (Fig. 1E). *B. ischaemum* had a curvilinear response in 1999 and a linear response in 2000 (Fig. 1F), while *B. japonicus* showed linear declines on both measurement dates (Fig. 1D). *S. dimidiatum* had the highest overall g_s of the three species in both years, followed by *B. japonicus* and *B. ischaemum*, respectively. Since g_s declined and A increased with CO_2 , intrinsic leaf water-use efficiency (A/g_s) increased linearly with C_a for all species in both years ($P < 0.05$, Fig. 2, Table 2). A slight curvilinear increase with C_a was observed for *S. dimidiatum* in 2000, but as this was driven largely by one outlier (shown in Fig. 2B), data were fit with a linear model.

The C_3 species showed significant linear declines in the ratio of intercellular to atmospheric CO_2 concentration

(C_i/C_a) with increasing C_a in both years ($P < 0.05$, Table 2). There was no consistent pattern in the direction of C_i/C_a changes with C_a for *B. ischaemum*.

Photosynthetic acclimation and light responses

There was evidence of up-regulation of A at subambient C_a for *S. dimidiatum*. The light saturated rate of carboxylation (V_{cmax}) calculated from A/C_i curves decreased significantly with increasing C_a up to concentrations approaching current ambient (Fig. 3A, Table 3). However, superambient C_a had no effect on V_{cmax} , suggesting acclimation to high C_a did not occur for this species. The values for maximum electron transport rate (J_{max}) for *S. dimidiatum* were variable and not significantly related to C_a concentration (Fig. 3B, Table 3). The ratio of electron transport capacity to carboxylation rate (J_{max}/V_{cmax}) provides an indicator of N allocation to different components of the photosynthetic process in the leaf (Medlyn 1996). In *S. dimidiatum*, J_{max}/V_{cmax} increased significantly from subambient to ambient C_a and did not change over superambient C_a (Fig. 3C, Table 3), suggesting proportionally greater allocation of N to Rubisco at subambient C_a . For *B. japonicus*, V_{cmax} , J_{max} , and J_{max}/V_{cmax} were highly variable and showed no significant relationship with C_a concentration (Fig. 3D–F, Table 3).

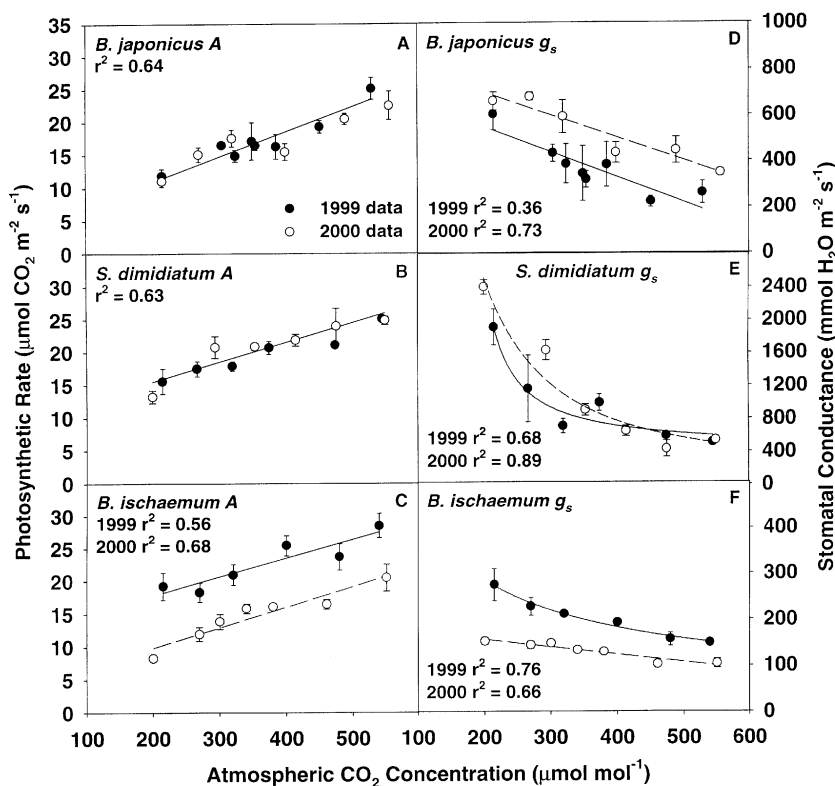


Fig. 1 Net photosynthetic (A) and stomatal conductance (g_s) rates for three species along a gradient of C_a concentrations in 1999 and 2000. Note the change in the conductance scale for the different species. Open symbols and dashed regression lines represent 2000 data. Data in panels A and B are plotted with a single line because regression results for the 1999 and 2000 data were very similar. See Table 2 for complete analysis results. Combined 1999/2000 regression results for panels A and B, respectively: parameter = 0.0308, intercept = 5.70, $P < 0.0001$; parameter = 0.0279, intercept = 9.82, $P < 0.0001$.

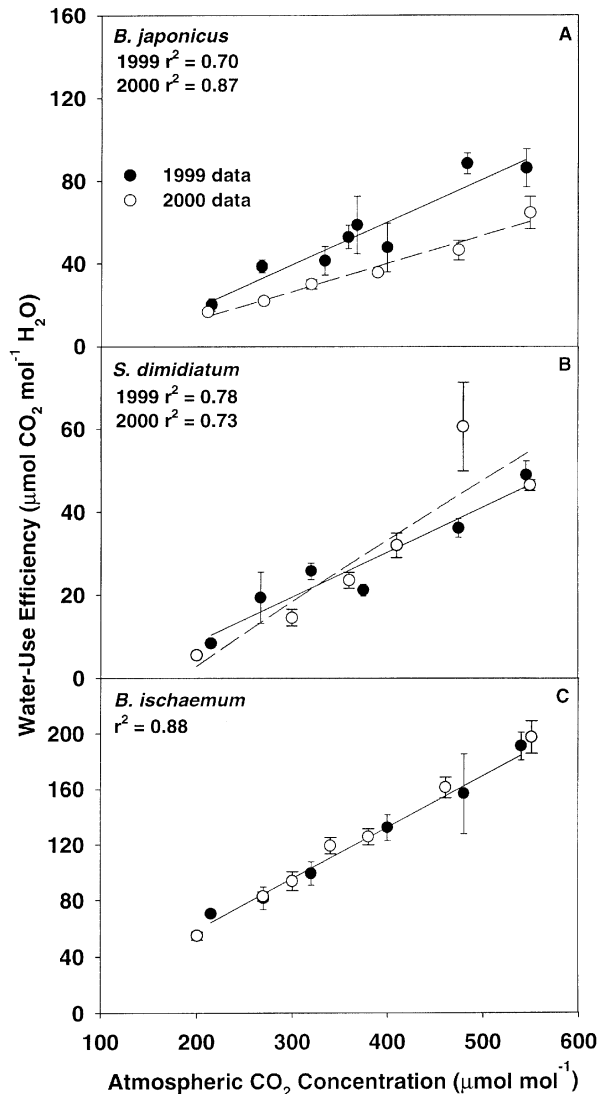


Fig. 2 Intrinsic leaf water-use efficiency (A/g_s) for three species along a gradient of C_a concentrations in 1999 and 2000. Note the change in the y-axis scale for the different species. See Fig. 1 legend for descriptions of panels and symbols, and Table 2 for analysis results. Combined 1999/2000 regression result for panel C: parameter = 0.3911, intercept = -21.48, $P < 0.0001$.

Although data were variable, there was a significant decrease in PEP carboxylase efficiency with C_a concentration for *B. ischaemum* (Fig. 3G, Table 3). CO₂ saturated photosynthesis (A_{max}) did not vary significantly with C_a concentration (Fig. 3H), but plants exposed to subambient C_a concentrations generally reached A_{max} at lower C_i concentrations than plants exposed to superambient C_a (Fig. 3I).

B. japonicus showed a significant linear increase in apparent quantum yield with C_a (Fig. 4A, Table 3).

S. dimidiatum and *B. ischaemum* showed linear increases in quantum yield with C_a up to 320 and 400 $\mu\text{mol mol}^{-1}$, respectively, and then the response levelled off for both species (Fig. 4B, C, Table 3). Thus, quantum yield did not change significantly over superambient C_a concentrations for either *S. dimidiatum* or *B. ischaemum*, even though superambient C_a had strong effects on light-saturated A in these same plants.

Leaf N and PNUE

All species showed significant or marginally significant decreases in leaf N content per unit area with increasing C_a in 1998 (Fig. 5, Table 4). Data were more variable in 1999: *B. japonicus* showed a linear decrease and there was no significant relationship between leaf N and CO₂ for *B. ischaemum* or *S. dimidiatum*. As leaf N generally decreased and A increased with CO₂, PNUE increased significantly with CO₂ for all species in 1999 (Fig. 6, Table 4).

Discussion

The goal of our study was to characterize leaf gas exchange responses of C₃ and C₄ grassland species to a continuous C_a gradient from past to future concentrations. One of our most important findings was an increased maximum rate of carboxylation (V_{cmax}) at subambient C_a for the C₃ perennial forb *S. dimidiatum*. To our knowledge, this is the first field study to document up-regulation of A in response to low C_a . In C₃ plants, light-saturated A is limited by V_{cmax} and by the regeneration rate of RuBP. The V_{cmax} limitation occurs at low C_i , while RuBP regeneration is limited by the maximum electron transport capacity (J_{max}) at intermediate C_i , and by inorganic phosphate P_i regeneration at high C_i (Sage 1994). It has been proposed that plants adjust N allocation among the biochemical components of A in response to changing C_a , devoting less N to carbon fixation and more to RuBP regeneration as C_a increases (Sage 1990; Sage 1994). Medlyn (1996) predicted that such N re-allocation should result in a 40% increase in J_{max}/V_{cmax} with a doubling of C_a . We found that J_{max}/V_{cmax} in *S. dimidiatum* increased by 37% as C_a increased from 200 to 400 $\mu\text{mol mol}^{-1}$ (Fig. 3, Table 3). *B. ischaemum* at subambient C_a also showed increased PEP carboxylase efficiency and reached maximum A at a lower C_i , suggesting up-regulation of A in this species as well (Fig. 3, Table 3). Assuming the activation state and specific activity of Rubisco remain constant (Sage *et al.* 1989), our data suggest that N re-allocation within the photosynthetic system does occur with C_a increases across subambient concentrations. This implies that significant photosynthetic adjustments may have taken place as C_a rose in the geological past. P_i regeneration

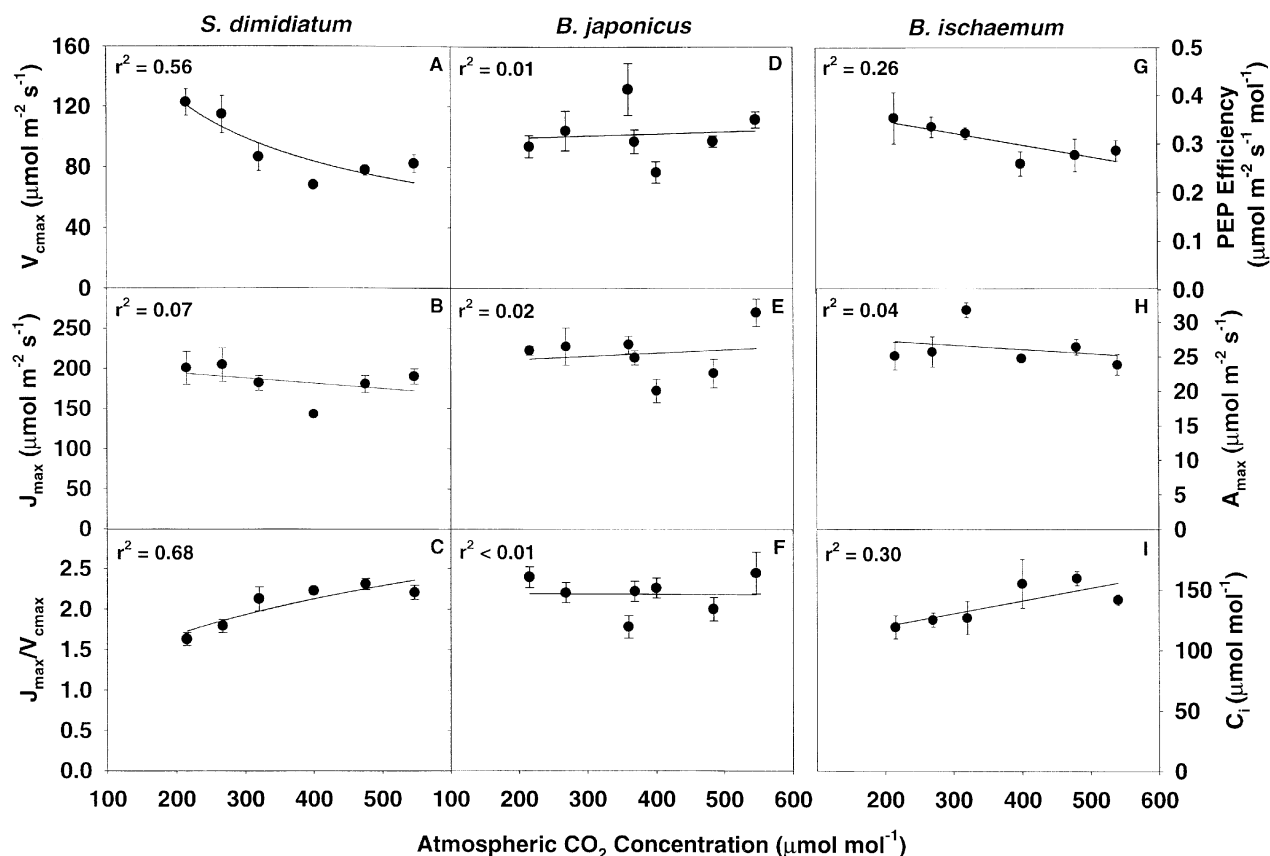


Fig. 3 Photosynthetic acclimation parameters for three species along a C_a gradient in 1999: (A, D) Maximum rate of Rubisco carboxylation (V_{cmax}) for the C_3 species *S. dimidiatum* and *B. japonicus*; (B, E) maximum electron transport rate (J_{max}) for *S. dimidiatum* and *B. japonicus*; (C, F) ratio of maximum electron transport rate to maximum carboxylation rate (J_{max}/V_{cmax}) for *S. dimidiatum* and *B. japonicus*; (G) efficiency of the PEP carboxylase CO_2 pump for the C_4 grass *B. ischaemum*; (H) maximum CO_2 -saturated A rate for *B. ischaemum*; and (I) C_i concentration at which A reached saturation for *B. ischaemum*. See Table 3 for analysis results.

limitations on A were not estimated in our study, but are not likely to influence A strongly at low C_a (Stitt 1991).

Our data contrast with previous studies of acclimation to subambient C_a , which did not find strong evidence for photosynthetic adjustments (Overdieck 1989; Thomas & Strain 1991; Tissue *et al.* 1995; Sage & Coleman 2001). To our knowledge, only three studies have found evidence of photosynthetic acclimation at low C_a . Sage & Reid (1992) and Cowling & Sage (1998) found that A is limited by P_i regeneration when *Phaseolus vulgaris* plants grown at subambient C_a were exposed to superambient C_a ; and Gesch *et al.* (2000) observed increased Rubisco small subunit gene expression in *Oryza sativa* L. exposed to subambient C_a . Different acclimation responses among studies could be explained in the context of carbon source-sink dynamics. Several authors have suggested that down-regulation of A at superambient C_a results from sink limitation, such as occurs when roots are restricted (Thomas & Strain 1991; reviewed by Sage 1994)

or when other resources are limiting (Curtis 1996; Bryant *et al.* 1998; but see Huxman *et al.* 1998). Exposure to subambient C_a in a field system such as ours may favour up-regulation of A in some species due to large sink demands coupled with substrate limitation of A .

Up-regulation of A may be related to increases in leaf N content, as well as to re-allocation of N within leaves. As Rubisco is a significant N investment, Sage & Reid (1992) predicted that large increases in leaf N would be needed for *P. vulgaris* grown at $200 \mu\text{mol mol}^{-1} C_a$ to achieve A equal to plants grown at $350 \mu\text{mol mol}^{-1}$. We found significantly higher leaf N (g m^{-2}) in all species at subambient C_a concentrations in 1998 (Fig. 5, Table 4), which could be additional evidence for up-regulation in *S. dimidiatum* and perhaps *B. ischaemum*. Leaf thickening in plants at high C_a could also create this trend, but no differences in leaf mass per unit area were found along the gradient for any species (L. J. Anderson and H. Maherali, unpublished data). In addition, direct meas-

Table 3 Regression analysis results on the relationship between C_a concentration and photosynthetic acclimation and light response parameters. Abbreviations are described in the legend of Table 2.

Variable, species and measurement date	Model type	Increase or decrease with C _a	Parameter value (a)	Intercept (b)	r ²	P-value
Photosynthetic acclimation parameters for <i>B. japonicus</i> in Apr 1999 and <i>S. dimidiatum</i> in Jul 1999						
V _{cmax}						
<i>B. japonicus</i>	Linear	NS	0.0139	96.31	0.01	0.7619
<i>S. dimidiatum</i>	Power	Decrease	-0.5638	2420.5	0.56	0.0005
J _{max}						
<i>B. japonicus</i>	Linear	NS	0.0410	203.28	0.02	0.5940
<i>S. dimidiatum</i>	Linear	NS	-0.05340	207.90	0.07	0.3385
J _{max} /V _{cmax}						
<i>B. japonicus</i>	Linear	NS	-0.00004	2.20	0.00	0.9578
<i>S. dimidiatum</i>	Power	Increase	0.3766	0.22	0.68	0.0001
Photosynthetic acclimation parameters for <i>B. ischaemum</i> in Aug 1999						
PEP carboxylase efficiency	Linear	Decrease	-0.00024	0.40	0.26	0.0320
A _{max}	Linear	NS	-0.00611	28.53	0.04	0.4059
Internal CO ₂ concentration	Linear	Increase	0.10574	98.56	0.30	0.0195
Quantum yield						
<i>B. japonicus</i> , Apr 2000	Linear	Increase	0.00005	0.0356	0.61	0.0001
<i>S. dimidiatum</i> , Jun 1999	Power	Increase	0.3613	0.0065	0.40	0.0091
<i>B. ischaemum</i> , Aug 1999	Power	Increase	0.5378	0.0023	0.62	0.0001

urements of soil bulk N in the experimental chambers did not show a strong underlying pattern (R. Gill, unpublished data), suggesting that leaf N levels are driven by plant and soil responses to the C_a gradient itself.

We found little evidence for photosynthetic acclimation (i.e. down-regulation) at superambient C_a. Photosynthesis showed a significant linear increase with C_a for all species in 1999 and 2000 (Fig. 1, Table 2), indicating that sensitivity to the C_a gradient persisted even after 3 years of CO₂ exposure. V_{cmax} and J_{max} parameters did not show acclimation to high C_a in any species (Fig. 3, Table 3). These data are consistent with many other field studies showing no A down-regulation and no photosynthetic adjustment at superambient C_a (Sage 1994; Curtis & Wang 1998; Medlyn *et al.* 1999; Herrick & Thomas 2001).

An important focus of our work was to compare plant physiological responses to increases over sub- and superambient C_a. Responses were strongly linear for A, indicating that A was affected similarly by C_a increases over the full range of concentrations. However, responses were often curvilinear for g_s, with steeper declines over subambient C_a (Fig. 1, Table 2). These data suggest that A for *B. japonicus*, *S. dimidiatum* and *B. ischaemum* may have been increasing with C_a over

geological time and will likely be sensitive to future C_a increases, which has important implications for biomass accumulation and ecosystem CO₂ fluxes in this grassland (Mielnick *et al.* 2001). However, g_s was more sensitive to increases over sub- than superambient C_a in the perennial species, such that future increases in C_a may not influence g_s as strongly as did the past rise in C_a. Reductions in g_s at superambient C_a have been linked with increased leaf water-use efficiency, plant water potentials and soil water content (e.g. Field *et al.* 1995; H. W. Polley, unpublished data). High g_s at subambient C_a may have influenced these parameters in the geological past, with low C_a exacerbating the effects of drought and other environmental stresses on plants (Polley *et al.* 1993; Cowling & Sage 1998; Sage & Cowling 1999). This may complicate reconstructions of past vegetation and climate patterns, which assume that species' physiological tolerances for arid conditions are similar to those observed today (Cowling & Sykes 1999).

Curvilinear responses in g_s also raise questions about the C_a levels to which plants are adapted. Sage & Cowling (1999) proposed that extant plants are still adapted to the low C_a conditions of the past, such that their responses to future C_a increases could be constrained. The relative lack of sensitivity of g_s to superambient C_a in our perennial species is consistent with

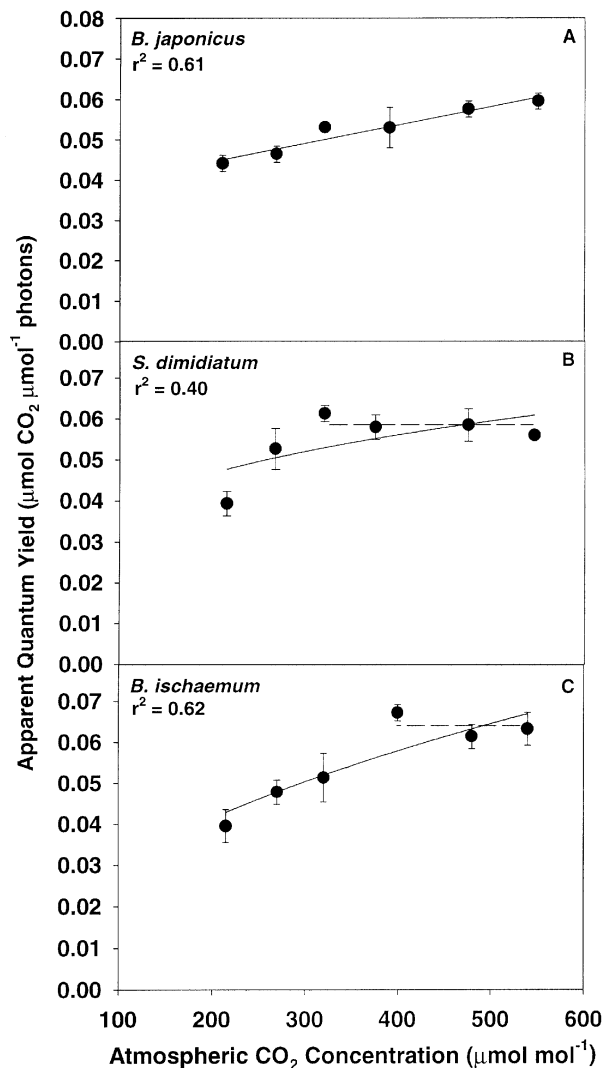


Fig. 4 Apparent quantum yields for three species along a gradient of C_a concentration in 1999 (*S. dimidiatum* and *B. ischaemum*) and 2000 (*B. japonicus*). See Table 3 for analysis results. Dashed lines in panels B and C were hand-fit to emphasize the flattening of the responses at superambient CO_2 that are not well described by the power functions fit to the full data sets.

this idea, but increased A at superambient C_a and the linear response of g_s to C_a in *B. japonicus* are not. Traits vary in their sensitivity to C_a and their degree of genetic variability (e.g. Curtis *et al.* 1996; Case *et al.* 1998; Ward *et al.* 2000), such that the selective pressure of C_a on A and g_s may differ among species and genotypes. Environmental conditions may also affect the strength of C_a as an agent of selection. Occasional changes in the shape of the g_s response curve over the 2 years in this study (Fig. 1, Table 2) imply that effects of C_a on leaf physiology may be modulated by resource availability.

Like many studies at superambient C_a (reviewed in Drake *et al.* 1997), we found that increasing C_a enhanced leaf-level resource-use efficiency. The combination of decreasing leaf N and increasing A at higher C_a concentrations led to linear increases in PNUE for all species in 1999 (Figs 1, 5, 6, Table 4). Water-use efficiency also increased linearly with C_a for all three species in both years (Fig. 2, Table 2), similar to findings by Polley *et al.* (1993, 1995) for C_3 plants grown in a subambient C_a gradient in greenhouse studies. Because the C_i/C_a ratio decreased with increasing C_a for the C_3 species in this study (Table 2), A/g_s of C_3 species increased proportionally more than did C_a ($A/g_s = C_a(1 - C_i/C_a)/1.6$). For example, a 33% increase in C_a from 270 to 360 $\mu\text{mol mol}^{-1}$ resulted in a 56% increase in A/g_s for *B. japonicus* in 1999. Sage (1994) observed that C_i/C_a often decreases at superambient C_a under drought or humidity stress, and suggested that stomata will become more conservative under water stress at future C_a levels. The dry conditions in our summer field experiment may explain why we saw declines in C_i/C_a , in contrast to the near-constant C_i/C_a ratios observed across C_a concentrations in several greenhouse studies (e.g. Sage & Reid 1992; Polley *et al.* 1993; Sage 1994; but see Tissue *et al.* 1995; Santrucek & Sage 1996). Many factors affect scaling of resource use efficiencies from leaves to canopies (Field *et al.* 1995), but the strong enhancements in PNUE and A/g_s observed here indicate the importance of past and future increases in C_a for productivity and resource balance of grasslands.

Leaf gas exchange data were also collected for the three study species along the C_a gradient in 1998, using the LI-6200 closed gas exchange system and ambient light (Li-Cor, Inc., Lincoln, NE, USA). The LI-6200 is inferior to the LI-6400 in its control of light, temperature and humidity in the leaf chamber, yet 1998 gas exchange trends were generally consistent with those seen in 1999/2000. In 1998, all species showed significant, generally curvilinear decreases in g_s with increasing C_a , as in 1999 and 2000 ($P < 0.01$ for all, data not shown). The C_3 species also showed increases in PNUE with C_a in May 1998 ($P < 0.05$, data not shown). One difference between 1998 and 1999/2000 was a less dramatic A response to increasing C_a for *S. dimidiatum* and *B. ischaemum*. Photosynthesis increased with C_a for both species, but the data were more variable than in 1999/2000, and not statistically significant. This may reflect the poorer environmental control of LI-6200 compared to the LI-6400, or the fact that measurement light levels in 1998 were well below saturation for the perennial species ($\geq 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, see also Table 1). Non-saturating light levels can reduce responses to C_a , particularly in C_4 plants (Sionit & Patterson 1984). Despite the reduced A response in 1998, A/g_s increased linearly with increasing C_a for all three species ($P < 0.01$, data not shown).

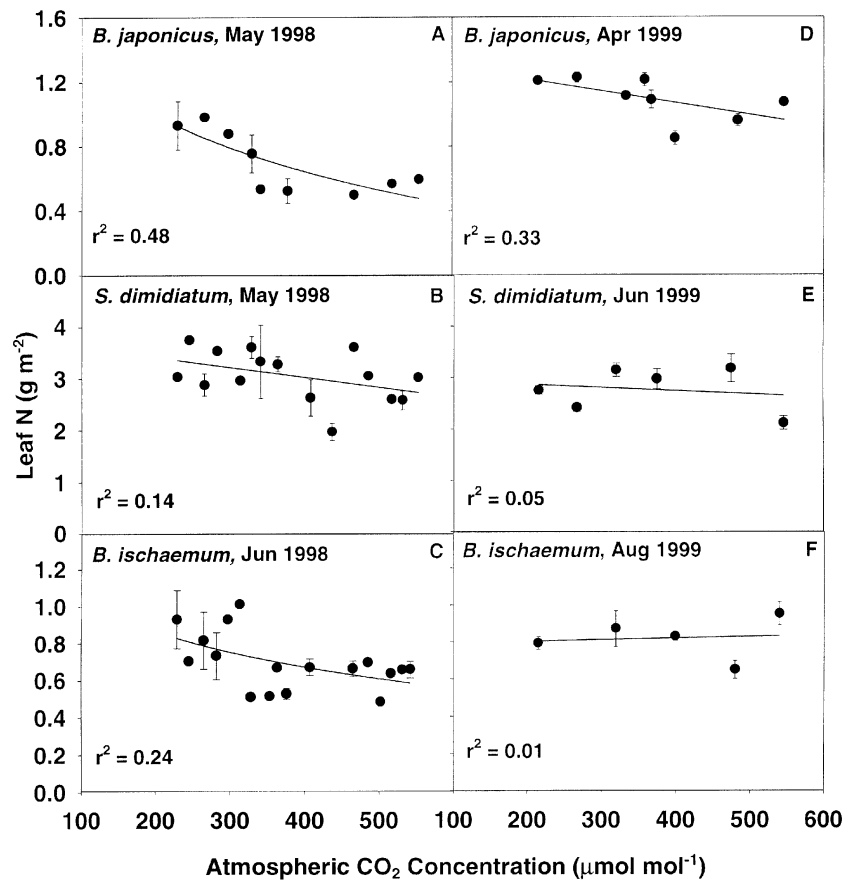


Fig. 5 Leaf N content for three species along a gradient of C_a concentration in 1998 and 1999. Note the change in the y-axis scale for the different species. See Table 4 for analysis results.

Table 4 Results of regression analyses on the relationship between C_a concentration and leaf N content for three species in 1998 and 1999 and photosynthetic nitrogen-use efficiency for three species in 1999. Abbreviations are described in the legend of Table 2.

Variable, species and measurement date P-value	Model type	Increase or decrease with C _a	Parameter value(s)	Intercept (a)	r ² (b)	P-value
Leaf N content (g m ⁻²)						
<i>B. japonicus</i> , May 1998	Log	Decrease	-0.4771	3.50	0.48	0.0060
<i>B. japonicus</i> , Apr 1999	Linear	Decrease	-0.0008	1.39	0.33	0.0062
<i>S. dimidiatum</i> , May 1998	Linear	NS	-0.0023	3.89	0.14	0.0539
<i>S. dimidiatum</i> , Oct 1998	Linear	Decrease	-0.0020	3.59	0.18	0.0418
<i>S. dimidiatum</i> , Jun 1999	Linear	NS	-0.0008	3.03	0.05	0.4082
<i>B. ischaemum</i> , Jun 1998	Log	Decrease	-0.2904	2.41	0.24	0.0071
<i>B. ischaemum</i> , Sep 1998	Linear	Decrease	-0.0009	1.17	0.19	0.0175
<i>B. ischaemum</i> , Aug 1999	Linear	NS	0.00009	0.78	0.01	0.7812
Photosynthetic nitrogen-use efficiency (A/leaf N content)						
<i>B. japonicus</i> , Apr 1999	Linear	Increase	0.0391	1.39	0.78	< 0.0001
<i>S. dimidiatum</i> , Jun 1999	Linear	Increase	0.0144	2.14	0.55	0.0007
<i>B. ischaemum</i> , Aug 1999	Linear	Increase	0.0305	17.24	0.46	0.0054

Therefore, increases in A/g_s in 1998 were largely driven by changes in g_s along the C_a gradient, suggesting that under non-saturating light conditions (which occur

frequently in the field), A/g_s responses to increasing C_a may be mediated largely by stomatal closure, not A enhancement.

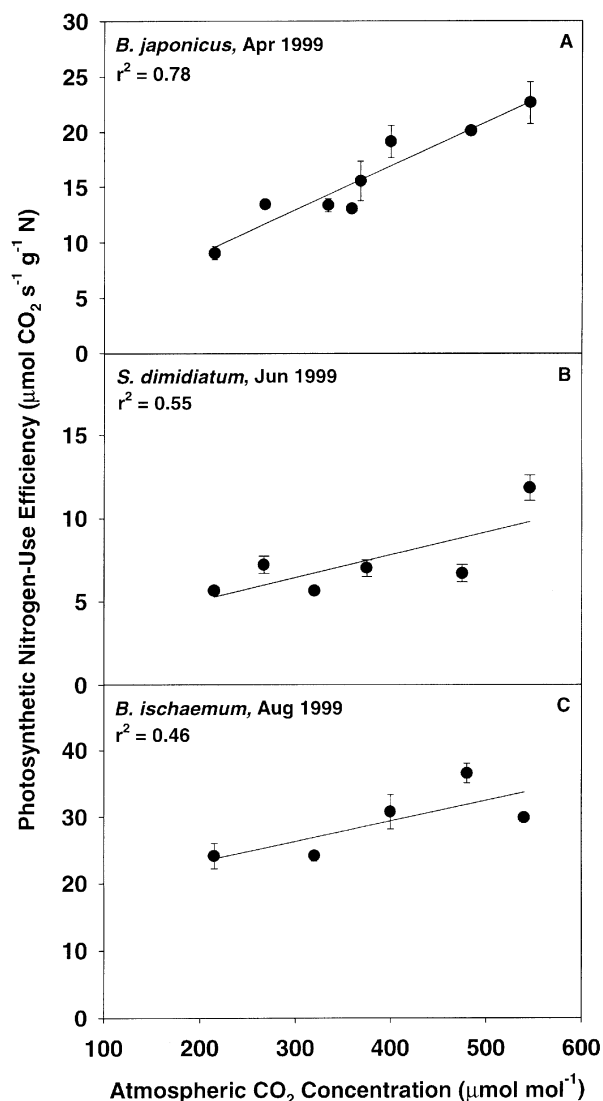


Fig. 6 Photosynthetic nitrogen-use efficiency ($A/\text{leaf N}$ content) for three species along a gradient of C_a concentration in 1999. Note the change in the y -axis scale for the different species. See Table 4 for analysis results.

Both the C_3 and C_4 species in this study responded to changes in C_a . Increasing C_a enhanced A slightly more in the C_3 than the C_4 species, yet A , A/g_s and PNUE increased, and g_s and leaf N decreased, significantly with increasing C_a for the C_4 grass *B. ischaemum* (Figs 1, 2, 5, 6). Others have also shown that C_4 grassland species respond to increased C_a with enhanced water-use efficiency and growth, and changes in tissue nutrient concentrations (e.g. Knapp *et al.* 1993; Owensby *et al.* 1993; Ghannoum *et al.* 2000). Decreases in PEP carboxylase content, bundle sheath cell wall thickness (Watling *et al.* 2000) and

A_{\max} (Morgan *et al.* 1994) have also been found in C_4 plants at high C_a , suggesting that C_4 species undergo photosynthetic acclimation. Our data add to a growing new perspective on the influences of C_a on C_4 plants. In a recent review, Wand *et al.* (1999) concluded that C_4 plants showed significant and consistent responses to C_a , often of a similar direction and magnitude to C_3 plants. Thus, rising C_a may not shift the competitive balance in favour of C_3 over C_4 plants to the extent once predicted.

Quantum yield has been suggested as a determinant of the relative dominance of C_3 and C_4 plants globally (e.g. Johnson *et al.* 1993; Ehleringer *et al.* 1997). The greater quantum yields of C_4 plants at high temperatures may allow them to dominate warm sites currently, while their CO_2 concentrating mechanism conferred a competitive advantage over C_3 plants at low C_a levels in the past (Ehleringer *et al.* 1997). C_a mediated increases in the quantum yield of C_3 plants (via suppression of photorespiration, Long & Drake 1991) are expected to favour C_3 over C_4 communities in the future. We found that quantum yield increased linearly with C_a across sub- and superambient concentrations for *B. japonicus*, but increased only over subambient concentrations for the other species. The lack of response in quantum yield to superambient C_a is surprising for the C_3 *S. dimidiatum*, although a similar pattern was found for *S. melonghena* by Bunce & Ziska (1999), who suggested that acclimation to high C_a can reduce quantum yield. Moreover, quantum yield did not differ between C_3 and C_4 species at a given C_a concentration, suggesting that at current temperatures, future C_a increases may not favour C_3 relative to C_4 plants in this grassland. Because the three species were measured on different dates (Table 1), our results may be affected by environmental conditions.

Physiologically based models are needed to improve our predictions of ecosystem responses to past and future C_a changes, but field tests of the relationships used in these models are often difficult, particularly for past C_a concentrations. We examined plant physiological responses over a gradient of C_a from past to predicted future levels, and found that responses are not necessarily linear. In addition, even tightly coupled responses, such as A and g_s , can have different response curves. We also found that acclimation to subambient C_a may modulate the slopes of these responses for some species, and that C_3 and C_4 plants are both strongly affected by C_a increases. These data emphasize the need to consider plant responses in natural communities over sub- through superambient C_a in order to scale effectively from leaves to ecosystems in past and future environments.

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